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PATENT & TRADEMARK OFFICE

PATENT
Attorney Docket No. 219482
DHHS Ref. No. E-144-96/2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Nelson et al.

Group Art Unit: 1632

Application No. 09/242,202

Examiner: Li, Quian J.

Filed: November 1, 1999

For: A NOVEL VECTOR FOR POLYNUCLEOTIDE VACCINES

RECEIVED
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OFFICE OF PETITIONS

DECLARATION UNDER 37 C.F.R. § 1.132 OF JERRY E. MANNING

Commissioner for Patents
Washington, D.C. 20231

I, Jerry E. Manning, hereby declare that:

1. I am Chair of the Department of Molecular Biology and Biochemistry at the University of California - Irvine and have been employed as such for nine years. I received my Ph.D. from the University of Utah in biochemistry and have been working in the field of biochemistry for thirty-two years. I have authored or co-authored numerous scientific journal articles, which are listed in my curriculum vitae, which is attached hereto.

2. I have read the subject patent application and believe that the teachings set forth therein reasonably enable a person having ordinary skill in the art to make humanized polynucleotide vectors comprising any known, human-derived promoter or mammalian homolog thereof any, human derived 3' splice sequence, and any human-derived poly A sequence.

In addition to those materials taught in the application, one of ordinary skill in the art would have known of other choices for the aforementioned promoters, splice sequences, and poly A sequences prior to August 14, 1996. For example, a myriad of human promoters were known in the art (see, for example, Ohlsson et al., *Int. J. Dev. Biol.*39(5): 869-876 (1995); and Forstermann et al., Naunyn Schmiedebergs Arch Pharmacol 352(4): 351-64 (1995)). Also, numerous 3' splice sequences were known in the art (see, for example, Blumenfeld

et al., *Hum. Mutat.* 6(3): 199-209 (1995); Tuchman et al., *Hum. Mutat.* 2(3): 174-8 (1993)). Additionally, methods for DNA vector construction were known in the art (see, for example, Molecular Cell Biology, Darnell, J. 248-262 (1986); Nischt et al., Eur. J. Biochem. 200(2): 529-536 (1991)). Moreover, on page 8, line 22, through page 22, line 21, the specification teaches how to construct a DNA vector using a human promoter and a human 3' splice sequence.

Using the specification as a guide, one of ordinary skill in the art would have been able to select an appropriate human promoter and an appropriate human 3' splice sequence and then construct a DNA vector in accordance with the teachings of the present invention. Choosing combinations of promoters, splice sites, and poly A sequences would have been a matter of routine laboratory experimentation. Furthermore, on page 22, line 22, through page 25, line 17, the specification provides guidance for using the constructed vectors. Additionally, the specification provides a framework for using the vectors to induce an immune response. It would have been a matter of routine to screen vectors for the ability to induce an immune response with a reasonable expectation of success. Since it is well-established that an antigen will induce an immune response in humans (see, for example, Fiore et al., *J. Gen. Virol.* 76 (Pt 8):1981-8 (1995)), one would have reasonably expected success in using the present inventive vector constructs to induce an immune response upon administration to humans.

3. I hereby declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 3/11/03

Jerry E. Manning, Ph.D.

CURRICULUM VITAE

Name: JERRY EDSEL MANNING

Date of Birth: October 19, 1944

Education:

University of Utah, Salt Lake City, Utah, B.Sc., 1966.

University of Utah, Salt Lake City, Utah, Ph.D., 1971.

Professional Experience:

Professor, Department of Molecular Biology and Biochemistry, University of California at Irvine, 1986 - Present. Departmental Chair 1994 - present.

Associate Professor, Department of Molecular Biology and Biochemistry, Department of Developmental and Cell Biology, University of California at Irvine, 1980-1986.

Assistant Professor, Department of Molecular Biology and Biochemistry, Department of Developmental and Cell Biology, University of California at Irvine, 1975-1980.

Research Fellow, Division of Chemistry and Chemical Engineering, California Institute of Technology, 1973-1974.

Research Fellow, Department of Biology, University of Utah, 1971-1972.

Awards:

NIH Training Grant Fellowship (Genetics) 1966-1967

NIH Training Grant Fellowship (Biochemistry) 1968-1971

Jane Coffin Childs Research Fellow 1973-1975

Research Career Development Award - NCI - 10/1/79-9/30/84

ASUCI Excellence in Teaching Award - 1997

ASUCI Excellence in Teaching Award - 1998

Society Membership:

American Association for the Advancement of Science, Elected Fellow

American Society for Biochemistry and Molecular Biology

American Association of Immunologists

The New York Academy of Sciences

Publications:

1. Richards, O.C., Ryan, R.S., and Manning, J.E. Effects of Cycloheximide and of Chloramphenicol of DNA Synthesis in Euglena gracilis, Biochem. Biophys. Acta 238, 190 (1971).

2. Manning, J.E., Wolsteinholme, D.R., Ryan, R.S., Hunter, J.A., and Richards, O.C., Circular Chloroplast DNA from Euglena gracilis, Proc. Nat. Acad. Sci., USA 68, 1169 (1971).

3. Manning, J.E., and Richards, O.C., Isolation and Molecular Weight of Circular Chloroplast DNA from Euglena gracilis, Biochem. Biophys. Acta 259, 285 (1972).

4. Manning, J.E., and Richards, O.C., Synthesis and Turnover of Euglena gracilis Chloroplast DNA, Biochemistry 11, 2036 (1972).

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6. Richards, O.C., and Manning, J.E., Replicating Chloroplast DNA Molecules in Euglena gracilis, Cycles Cellulairrs et Leur Blocage 213, (1974).

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8. Fouts, D., Manning, J.E., and Wolstenholme, D.R., Physical Characterization of Kinetoplast DNA of Crithidia acanthocephali, J. Cell. Biol. 67, 378 (1975).

9. Manning, J.E., Schmid, C., and Davidson, N., Interspersion of Repetitive and Non-repetitive DNA Sequences in the Drosophila melanogaster Genome, Cell 4, 141 (1975).

10. Schmid, C., Manning, J.E., and Davidson, N., Inverted Repeat Sequences in the Drosophila Genome, Cell 5, 159 (1975).

11. Manning, J.E., Hershey, N.D., Broker, T.R., Pellegrini, M., Mitchel, H.K., Davidson, N., A New Method of in situ

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14. Manning, J., Pellegrini, M.P., and Davidson, N., A Method for Gene Enrichment Based on the Avidin-Biotin Interaction. Application to the Drosophila Ribosomal RNA Genes, Biochemistry 16, 1364 (1977).

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19. Zimmerman, L., Fouts, D.L., and Manning, J., Evidence for a Complex Class of Nonadenylated Messenger RNA in Drosophila melanogaster, Genetics 95, 673 (1980).

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expression of the 85 kDa surface antigen gene among different strains of Trypanosoma cruzi. In "Molecular and Immunological Aspects of Parasitism", C.C. Wang, ed., American Association for the Advancement of Science, Washington, D.C., pp. 65-72 (1991).

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51. Ruef, B.J. and J. E. Manning. Isolation and Characterization of the TSA Family Member of the Silvio Strain of Trypanosoma cruzi. Mol. Biochem. Parasitol., 62, 117-120, (1994).

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65. Luhrs, K., Fouts, D. and J.E. Manning. Immunization and Recombinant Paraflagellar Rod Protein Induces Protective Immunity Against *Trypanosoma cruzi* Infection. Vaccine In Press.

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Review of Research Program and Grants:

a. National Science Foundation

b. NIH Study Section-Tropical Medicine and Parasitology - Member, 1987-1991

c. NIEHS-Program project reviews and site visits

d. NIH-Microbiology and Infectious Diseases Advisory Committee

e. Review Committee, UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, Member, 1986-1991

f. Swedish Agency for Research Cooperation with Developing Countries (SAREC) Consultant, 1991-present

g. International Scientific Advisory Board: Network for Research and Training in Parasitic Diseases at the Southern Cone of Latin American, Member, 1995-present

Editorial Service:

Editorial Board - Molecular and Biochemical Parasitology (1990 - 1994).

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PATENT & TRADEMARK OFFICE

#22

PATENT
Attorney Docket No. 219482
DHHS Ref. No. E-144-96/2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Nelson et al.

Group Art Unit: 1632

Application No. 09/242,202

Examiner: Li, Quian J.

Filed: November 1, 1999

For: A NOVEL VECTOR FOR POLYNUCLEOTIDE VACCINES

DECLARATION UNDER 37 C.F.R. § 1.132 OF EDWARD NELSON

Commisioner for Patents
Washington, D.C. 20231

I, Edward Nelson, hereby declare that:

1. I am an inventor of the subject matter disclosed and claimed in the above-identified application.

2. A person having ordinary skill in the art would have known of numerous human-derived 3' splice sequences, other than the specific 3' splice sequences disclosed in the above-identified application, before August 14, 1996. The following references are examples of prior art references that teach 3' splice sequences: Blumenfeld et al., *Hum. Mutat.* 6(3): 199-209 (1995) and Tuchman et al., *Hum. Mutat.* 2(3): 174-8 (1993).

3. Sustained expression of a humanized polynucleotide vector is unnecessary to elicit an immune response, as demonstrated below, and, in the context of the present invention, can be undesirable as persistent antigen is well known to elicit immune tolerance or anergy. Data (appended herein as Exhibit 1) were collected from an *in vitro* cellular proliferation assay. In this assay, splenocytes from pITL-rNeu immunized animals (immunized one every three weeks for a total of three immunizations) were

incubated for 5 days in media, alone (negative control), or with concavalin A (positive control), or with Rat 2 (specificity control), or with 13762 mammary tumor. Rat 2 is a syngeneic transformed fibroblast derived tumorigenic cell line that does not express rat neu whereas the syngeneic 13762 tumor modestly over expresses rat neu. Tritiated thymidine was added to the cultures for the final 18 hours and cells were harvested with incorporated ^{3}H thymidine determined by scintillation counting. These data support induction of antigen specific immunity by using this vector for an anti-tumor DNA immunization strategy.

Additionally, data (appended herein as Exhibit 2) depict results from an *in vivo* tumor challenge experiment. The data show results from three cohorts of animals (rats). Rats in cohort # 1 were untreated but similarly housed as rats from the other cohorts. Rats in cohort # 2 were immunized once every three weeks for a total of three immunizations with 100 μg of the pITL vector backbone (lacks target antigen). Rats in cohort # 3 were immunized once every three weeks for a total of three immunizations with 100 μg of the pITL-rNeu (expressing a tumor target antigen) vector backbone (lacks target antigen).

Eleven weeks into the experiment (two weeks after completion of the immunization sequence in cohorts 2 & 3) all animals received a tumor (13762) challenge. Animals were euthanized at a designated tumor volume without regard for treatment. This data shows that cohort # 3, rats immunized with pITL-rNeu, had significantly delayed tumor out growth relative to cohorts 1 and 2. This data supports the induction of anti-tumor immunity by this vector backbone expressing an appropriate tumor target antigen.

4. I hereby declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

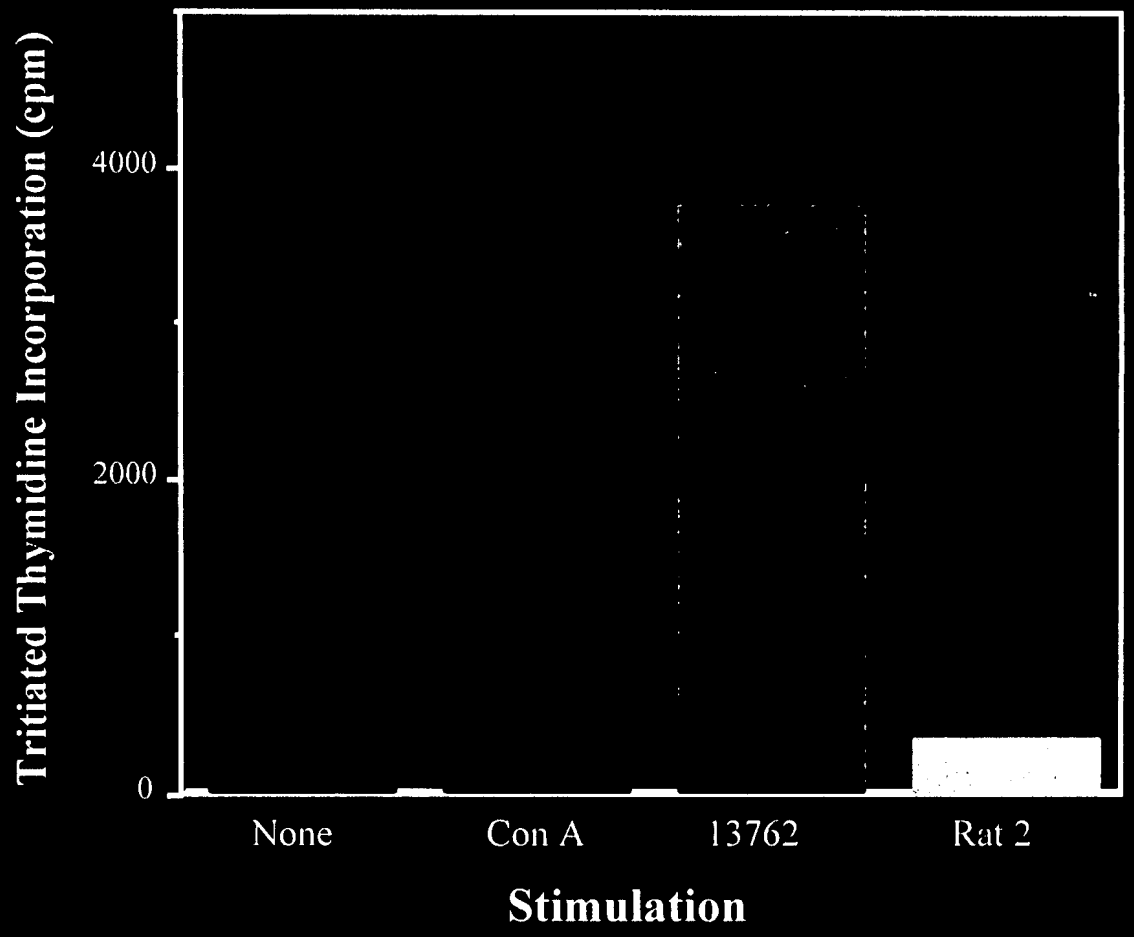
In re Appln. of Nelson et al.
Application No. 09/242,202

Date: 2/20/03

Edward Nelson, M.D.

EXHIBIT 1

Proliferation Assay



Values represent average of triplicate determinations and standard errors were less than 5% of the average value for each condition.

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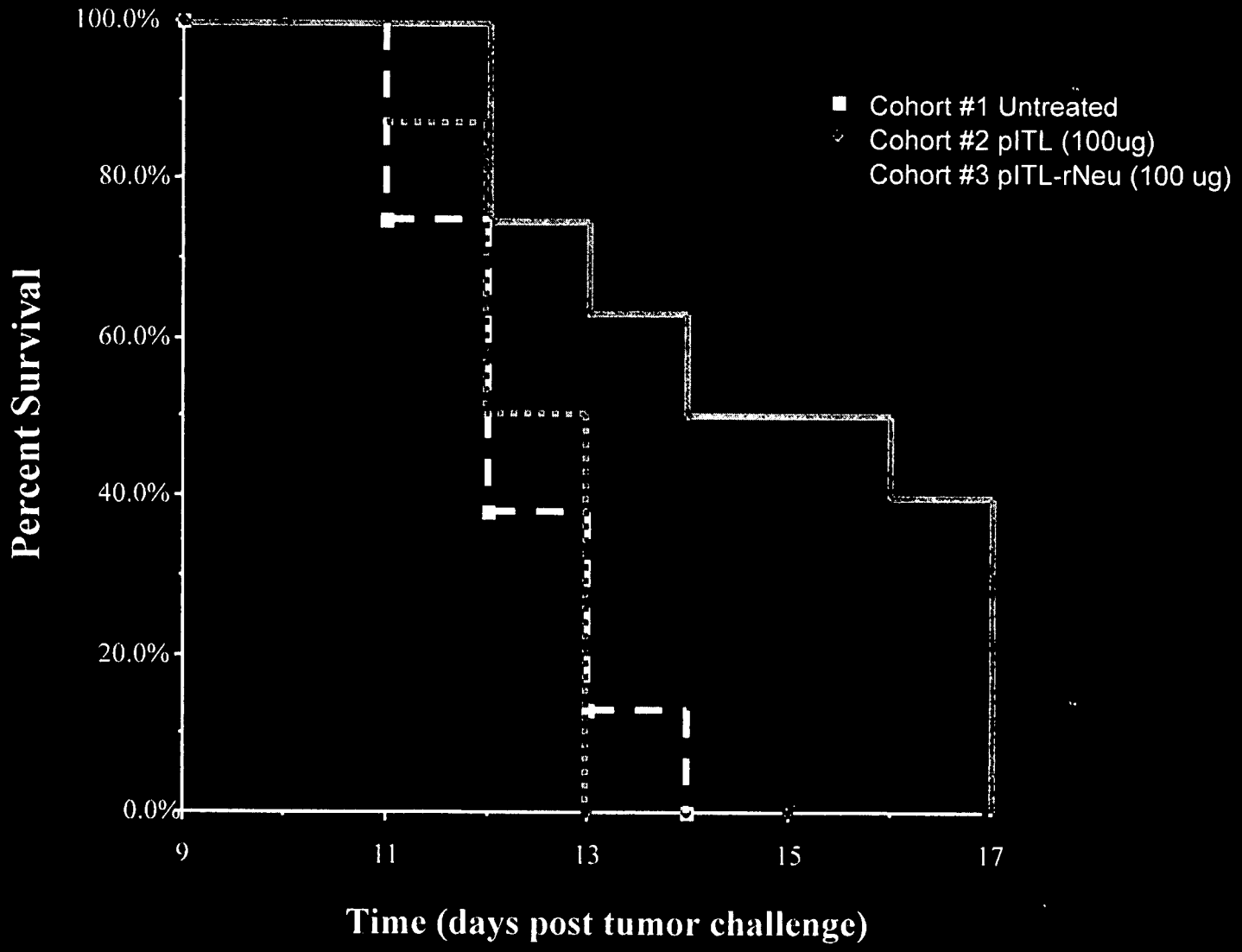
EXHIBIT 2

Anti-rat Neu PNV



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